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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/069,433	05/31/2002	Hans Kiefer	WWELL61.001APC	3712

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EXAMINER

HANLEY, SUSAN MARIE

ART UNIT	PAPER NUMBER
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1651

DATE MAILED: 09/22/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

10/069,433

Applicant(s)

KIEFER ET AL.

Examiner

Susan Hanley

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 31 May 2002.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-14 and 17-21 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-14 and 17-21 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All b) ☐ Some * c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
 - ☐ Certified copies of the priority documents have been received in Application No. _____.
 - ☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- ☒ Notice of References Cited (PTO-892)
- ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date 3 sheets.
- ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____.
- ☐ Notice of Informal Patent Application (PTO-152)
- ☐ Other: _____.

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DETAILED ACTION

Claims 1-14 and 17-21 are presented for examination.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 1, 5 and 14 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 3, 15 and 17 of copending Application No. 10/736,448. Claim 1 of the instant application is drawn to a method of making proteins from the G-protein-coupled receptors that have been folded into their native structure comprising the steps of solubilizing said protein in a first detergent and then exchanging the first detergent for a second detergent to induce folding into the active form. Claim 1 also specifies that the second detergent is an alkylglycoside, a glycoside or an alkyl phosphorylcholine. Claim 5 limit the exchange method to a chromatographic method. Claim 14 specifies that the second detergent is an alkyl phosphorylcholine with a chain length of 10-16 carbons.

Claim 3 of '448 is drawn to an invention that overlaps the scope of the instantly claimed invention because it specifies a method of preparing a refolded, recombinantly expressed eukaryotic membrane protein by solubilizing the protein in a first detergent, inducing refolding of the protein into its active form by exchanging the first detergent with a second detergent and then performing size exclusion chromatography of the refolded protein in solution. The specification of '488 discloses that a G-protein-coupled receptor is one type of a membrane protein (p. 10, section 0035). The specification of '488 also defines the scope of the "second detergent" to include alkyl glycosides having 5-12 carbons, alkyl phosphocholines having a chain length of 8-16 carbons or saccharide fatty acid esters (p. 14, section 0049). The specification can always be used as a dictionary to learn the meaning of a term in the patent claim. *In re Boylan*, 392 F.2d 1017, 157 USPQ 370 (CCPA 1968). Further, those portions of the specification

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which provide support for the patent claims may also be examined and considered when addressing the issue of whether a claim in the application defines an obvious variation of an invention claimed in the patent. In re Vogel, 422 F.2d 438, 441-42, 164 USPQ 619, 622 (CCPA 1970).

Claim 15 of '488 limits the second detergent to those claimed in claim 1 of the instant application. Claim 17 of '488 further define chromatographic methods for detergent exchange that falls within the scope of claims 3 and 5 of the instant application. The method of '488 comprises an additional step "c" that is not claimed in instant claim 1. However, the language of instant claim 1 is "open" (comprising) and encompasses additional steps. In conclusion, claims 3, 15 and 17 of '488 fall within the scope of the instantly claimed invention.

This is a provisional obviousness-type double patenting rejection.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-14 and 17-21 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is rejected because it is written in an improper Markush format and the use of "comprising" in a Markush claim is not consistent with Markush language. Alternative expressions are permitted if they present no uncertainty or ambiguity with respect to the question of scope or clarity of the claims. One acceptable form of alternative expression, which is commonly referred to as a Markush group, recites members as being "selected from the group consisting of A, B and C." See Ex parte Markush, 1925 C.D. 126 (Comm'r Pat. 1925). It is suggested that the 8th line of claim 1 be changed to read: "alkyl chain, [and] glycoside".

Claim 1 is rejected because the phrase "providing said protein solubilized in a first detergent is confusing. The "said protein" refers to the folded protein in its active form in the preamble. It is unclear if the claim is directed to refolding proteins that are already in their active state or if the claim is directed to the folding of unfolded, inactive proteins into folded proteins in their active state.

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Claims 7 and 18 are rejected because "at least one conserved disulfide bridge" lacks antecedent basis in claim 1.

Claim 9 is rejected because the phrase "said protein is produced in form of inclusion bodies" is vague and indefinite. The use of "in form" is confusing because it implies that the protein has mutated into an inclusion body as opposed to a situation wherein the protein accumulates and resides inside the inclusion body.

Claim 11 is rejected because it is confusing. It is unclear if the first detergent is added before, after or both before and after the solubilization step.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

Claims 1-3, 5, 6, 9-14 and 17-21 are rejected under 35 U.S.C. 102(a) as being clearly anticipated by Kiefer et al (December 1999) Biochem. Soc. Trans. 27(6): 908).

Kiefer et al. disclose the expression and refolding of fusion proteins comprising G-protein-couple receptors (GPCRs) into active structures by sequentially contacting unfolded proteins with two detergents, as required by claim 1. The GPCR was expressed in E. coli as a fusion protein with glutathione S-transferase (GST) and accumulated in inclusion bodies which meets the limitations of claim 9 wherein the protein is produced by an expression vector in a transformed cell line. The inclusion bodies were solubilized, as required by claim 1, by combining said inclusion bodies with 1.5% N-lauorylsarcosine in a bath sonicator, diluting with four volumes of PBS containing 0.2% Digitonin. This disclosure meets the limitations of claim 11 that require that the inclusion bodies are purified by combined with the first detergent (N-lauorylsarcosine) for solubilization. The addition of thrombin served to cleave the GST moiety from the fusion protein, which meets the limitation of claim 10. The final step of solubilization, chromatographing the receptor protein on a Ni-NTA agarose column, produced the solubilized aggregated receptor that was cleaved from the GST portion of the fusion protein. The receptor protein

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was refolded by combining the aggregated protein in a first detergent, N-laurylsarcosine. The disclosure of N-lauroyl sarcosine as the first detergent meets the limitation of claim 12. N-lauroyl sarcosine has a high critical micellar concentration which allows for subsequent removal by a second detergent by dilution, as in claim 6 or by chromatography, as in claim 5. The solubilized protein can be rapidly transferred into another detergent by diluting below the CMC of N-lauroyl sarcosine. When N-lauroyl sarcosine is below its CMC it becomes monomeric and the second detergent binds to the receptor protein. The second detergent can be Digitonin or lysolecithin. Lysolecithin is an alkyl-phosphorylcholine having a chain length of 16 carbons. This detergent meets the second detergent requirement of claims 1, 14 and 19-21 that require an alkyl phosphorylcholine of chain length C10-16. Lysolecithin meets the limitations of claims 2 and 3 which state that the second detergent must be from a natural source. Lysolecithin is provided with mixed lipid/detergent micelles, as required by claim 2 and a CAM above that of N-lauroyl sarcosine, thus satisfying the limitation of claim 13.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-6, 8-14, 17 and 10-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kiefer et al. (Biochem. (1996) 35: 16077) in view of Kiefer et al. (Biochem. Soc. Trans. (July 1999) A141) which will be referred to as Kiefer 1999 and Nekrasova et al. (Eur. J. Biochem. (1996) 238: 28).

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Kiefer et al. disclose the expression and refolding of an olfactory receptor protein that is a member of the G-protein-couple receptors by a two detergent method, as required in claim 1. The olfactory receptor was expressed in *E. coli* cells as a fusion protein with glutathione S-transferase. The fusion protein was overexpressed and accumulated in inclusion bodies. This disclosure meets the limitations of claims 9 and 10 which required cloning of the protein into a transformed cell line to form a fusion protein that accumulates in inclusion bodies. The inclusion bodies were isolated and solubilized in N-lauroyl sarcosine, the first detergent, in TBA (Tris buffered saline) in a bath sonicator. Digitonin was added and the mixture was centrifuged. The supernatant was loaded onto a Ni-NTA agarose column which as equilibrated with Digitonin and the supernatant was eluted with a histidine gradient. The fractions were pooled and dialyzed against Tris and DTT. Thrombin and N-lauroyl sarcosine were added and the sample was incubated for 15h to cleave off the GST moiety as required by claim 10. The cleaved protein in N-lauroyl sarcosine (the first detergent) was rechromatographed on Ni-NTA and eluted with Digitonin (the second detergent). Although Kiefer et al. do not expressly describe these steps as a refolding mechanism, the disclosed steps of solubilization in a first detergent and exchange of the first detergent by a second detergent by a chromatographic method meets the limitations of claims 1, 5, 11 and 12. The folded protein was then converted into a liposome by combining the refolded protein with egg phosphatidylcholine, phosphatidylglycerol, dodecyl and maltoside in chloroform to make a lipid layer around the refolded protein, which is by definition, a lipoproteosome, which meets the limitations of claim 8.

Kiefer et al. do not teach that the second detergent is an alkyl-phosphorylcholine having a chain length of 10-16 carbons or that the detergent exchange was accomplished by dilution, dialysis or ultrafiltration.

Kiefer et al. 1999 disclose that eleven different GPCRs were expressed in separate fusion proteins with GST in inclusion bodies. The majority of the fusion proteins were found to be insoluble in neutral detergents, indicating aggregation and partial or no insertion of the fusion protein in a membrane. The proteins were refolded in their native states by solubilizing the inclusion bodies with N-lauroyl sarcosine, proteolytically cleaving the GST portion and purification by an Ni chelating column chromatography. During purification N-lauroyl sarcosine was replaced by a milder detergent to initiate refolding. Kiefer et al. 1999 do not name a specific milder detergent.

Nekrasova et al. teach overexpression, solubilization and purification of rat and human olfactory receptors. Nekrasova et al. disclose that lysophosphatidyl choline is a better choice than N-lauroyl sarcosine for the

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solubilization and purification of olfactory receptors because it is milder and less likely to interfere with subsequent purification steps (p. 33, left column). Lysophosphatidyl choline is a C-16 alkyl phosphorylcholine that is found in eggs and meets the limitations of claims 1, 14, 17 and 19-21.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to employ lysophosphatidyl choline in the method taught by Kiefer et al. Kiefer et al. teach the refolding of an olfactory receptor, which is a G-protein-coupled receptor, by a sequential, two detergent method using N-lauroyl sarcosine and Digitonin. Kiefer et al. 1999 disclose the same method with the same type of olfactory receptor and recommend using a milder detergent for the second step of the refolding method. The ordinary artisan would have been motivated to employ lysophosphatidyl choline for olfactory receptor protein refolding because Nekrasova et al. specifically recommend it since it is mild and less likely to interfere with subsequent purification steps. The ordinary artisan would have had a reasonable expectation that lysophosphatidyl choline would be successful for olfactory receptor protein refolding because Nekrasova et al. demonstrated this.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to carry out the detergent exchange by dilution, dialysis or ultrafiltration in the method of Kiefer et al. The ordinary artisan would have known that chromatography, dilution, dialysis and ultrafiltration are standard, equivalent methods to eliminate unwanted components from a mixture. The ordinary artisan would have been motivated to choose the method, chromatography, dilution, dialysis or ultrafiltration, to accomplish the detergent exchange in the most efficient and least expensive manner.

Allowable Subject Matter

Claims 7 and 18 would be allowable if rewritten to overcome the rejection(s) under 35 U.S.C. 112, 2nd paragraph, set forth in this Office action and to include all of the limitations of the base claim and any intervening claims.

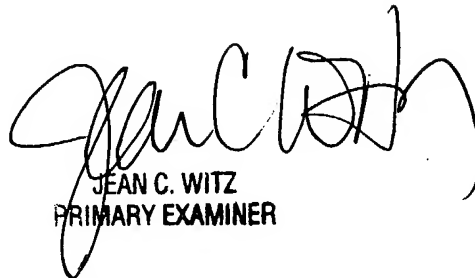
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Any inquiry concerning this communication or earlier communications from the examiner should be directed to Susan Hanley whose telephone number is 571-272-2508. The examiner can normally be reached on M-F 9:00-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Susan Hanley
Examiner
1651



JEAN C. WITZ
PRIMARY EXAMINER